



Effect of Different Durations of Shawkea DE-T1 Administration on Blastocyst Obtained Rate in Women Receiving IVF-ET Treatment: A Secondary Analysis of a Cohort Study

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ABSTRACT

Objective: To explore the appropriate duration of Shawkea DE-T1 use, and to provide a basis for the optimization of the Shawkea DE-T1 administration duration for different women.

Methods: Based on a previous retrospective cohort study, 1,014 patients aged ≥ 30 years who used *In vitro* Fertilization (IVF) for conception at Hanabusa Women's Clinic, Kobe, Japan, were included in this secondary analysis and were allocated to an Shawkea DE-T1-administration group (n=712) and a control group (n=302) based on their use of Shawkea DE-T1. All patients in the two groups received interventions following the guidelines of the Japanese Institution for Standardizing Assisted Reproductive Technology Intervention, and patients in the administration group were provided Shawkea DE-T1 as recommended by the Nutritional Supplement Support Center of Hanabusa Women's Clinic. The blastocyst obtained rate (percentage of patients who produced at least one blastocyst upon *in vitro* embryo culture relative to all patients in the same group) was compared between the two groups of patients following treatment durations of 1-3 months, 4-6 months, and >6 months. Analysis was performed on the actual duration of Shawkea DE-T1 administration for all patients who achieved blastocyst *in vitro* according to their age level (≥ 30 and <35 years of age; ≥ 35 and <40 years; ≥ 40 and <43 years; and ≥ 43 years of age).

Results: After a Shawkea DE-T1 administration of 1-3 months or 4-6 months, the blastocyst obtained rates in the administration group were significantly higher than those of the control group (83.27% vs. 55.31% for 1-3 months, $P=1.02 \times 10^{-10}$; 69.44% vs. 44.44% for 4-6 months, $P=4.70 \times 10^{-4}$), while no significant difference was uncovered between the two groups with >6 months of administration (73.35% vs. 72.46%, $P=0.76$). Analysis of the treatment duration of patients at different age levels who produced blastocysts showed that the treatment duration increased commensurate with patient age: i.e., 65.25% of women ≥ 30 and <35 years of age achieved blastocyst after a Shawkea DE-T1 administration of 1-3 months; while only 19.75% of women ≥ 43 years of age successfully achieved *in vitro* development of embryos to blastocyst stage with a Shawkea DE-T1 administration of 1-3 months.

Conclusion: Shawkea DE-T1 use for 1-3 months and 3-6 months significantly improved the blastocyst obtained rate in women receiving IVF treatment. Appropriate extension of Shawkea DE-T1 administration duration also achieved a better effect in women of advanced reproductive age.

Keywords: Shawkea DE-T1; Blastocyst; Live-birth rate; Fertilization; Infertility

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INTRODUCTION

A woman's fertility certainly declines with age, but the age at marriage and childbearing in modern society has also been delayed. This only intensifies associated anxieties, narrows the fertile timeline, and augments the infertile female population. In addition, the number of intractable infertilities has been gradually increasing. The purpose of Assisted Reproductive Technology (ART) is to collect numerous mature oocytes for *In vitro* Fertilization (IVF) by the common use of gonadotropins to stimulate the ovaries. However, many women of an advanced reproductive age and with infertility, or women with low ovarian function, are incapable of producing high-quality oocytes after ovulation induction due to poor ovarian response reducing the likelihood of culturing the fertilized oocyte to blastocyst stage, or of experiencing a successful pregnancy that results in a live birth. Therefore, successful retrieval and subsequent culture of the fertilized oocytes to embryos (blastocysts) is of great significance for successful pregnancy following *In vitro* Fertilization and Embryo Transfer (IVF-ET).

Dandelion (*Taraxacum mongolicum* Hand-Mazz.) is a traditional Chinese medicine with a long history and is used extensively in Japan and China, and there have been exciting developments in the area of dandelion polysaccharide research in recent years. Many investigators have demonstrated that dandelion polysaccharide exerts multiple functions that include antioxidant, anti-free radical, hepatoprotective, hypoglycemic, growth-factor secretory, and antitumor activities [1-4]. The principal raw material of Shawkea DE-T1 is dandelion, and its primary component is a type of amino-polysaccharide extracted from dandelions. Our previous study ascertained that Shawkea DE-T1 regulated the pituitary-ovarian reproductive axis and female hormone secretion, and promoted growth and inhibited apoptosis of ovarian granulosa cells [5,6]. Shawkea T-1 (Tokujun Co., Ltd), with the essential component DE-T1, is an assisted reproductive supplement which has been used in Japan for 24 years, and for over 10 years in China.

In our previous report we implemented a cohort study in which we discerned the effects of Shawkea DE-T1 combined with ovulation-induction drugs on blastocyst generation from *in vitro* culture of fertilized oocytes and on live births attained in women after IVF-ET [7]. Our results showed that women administered Shawkea DE-T1 in IVF achieved a 75.98%blastocyst obtained rate (541/712) that was significantly higher than women who did not take Shawkea DE-T1 (57.28%, 173/302; odds ratio=2.36, $P=2.4 \times 10^{-9}$). In addition, the blastocyst obtained rates in the Shawkea DE-T1-administration group for different age groups (≥ 30 and <35 years of age; ≥ 35 and <40 ; ≥ 40 and <43 ; and ≥ 43 years old) and that expressed various Anti-Mullerian Hormone (AMH) values ($AMH \leq 1.1$, $AMH >1.1$) were also significantly elevated relative to values in the control group ($P<0.05$).

For patients who underwent ET, the live-birth rate in the Shawkea DE-T1-administration group was 57.53% (107/186) compared to the control group (40.00%, 18/45; odds ratio=1.32, $P<0.05$), providing clinical evidence to support the application of Shawkea DE-T1 in infertility. In addition, another study revealed that the treatment duration for women who received IVF treatment and obtained blastocysts or live births varied greatly, and age was a critically important factor [8]. To optimize the use of Shawkea DE-T1, we herein evaluated the effect of Shawkea DE-T1 on blastocyst obtained rate by different administration durations. Furthermore, we summarized and analyzed the use of Shawkea DE-T1 among women who consumed Shawkea DE-T1, and successfully obtained blastocysts after IVF in the real world, to determine the optimal Shawkea DE-T1 administration duration so as to generate favorable results for infertile women at different ages. We posit that this will provide a basis for the optimal use of Shawkea DE-T1 in female infertility. This study was approved by the Ethics Committee of the Hanabusa Women's Clinic, Kobe, Japan.

Inclusion criteria

We here in included all Chinese women who traveled to Japan for IVF treatments at the Hanabusa Women's Clinic from August 1, 2012, to February 29, 2020 and who were ≥ 30 years of age; we did not limit inclusion based on their cause of infertility (poor ovarian function, polycystic ovary syndrome, uterine fibroids, adenomyosis, or blocked fallopian tube) or underlying diseases. All patients signed an informed-consent form before participating in the study and cooperated with data collection.

Exclusion criteria

Patients with incomplete general information or who became pregnant naturally during the treatment period were excluded from our study.

Overall condition of the patients

During the observation period, from August 1, 2012 to February 29, 2020, a total of 1,014 Chinese women who received IVF treatment at the Hanabusa Women's Clinic in Japan were included in this study. All patients were asked whether they would voluntarily receive Shawkea DE-T1. 712 patients who chose to take Shawkea DE-T1 were included in the administration group, with an administration duration ranging from 1 to 40 months and with a median (Q25, Q75) of three (0,6) months. Patients who decided not to undergo Shawkea DE-T1 administration were included in the control group. We noted no significant differences in the patients' general condition, age of the spouse, or IVF treatment plans between the two groups of patients ($P>0.05$), observed in Table 1.

	Administration group		Control group		P
	Cases		Cases		
Age (years) (mean $\pm$ SD)	712	38.50 $\pm$ 4.57	302	38.24 $\pm$ 4.83	0.41
≥ 30 and <35	155	32.28 $\pm$ 1.38	74	31.95 $\pm$ 1.51	0.1
≥ 35 and <40	250	37.00 $\pm$ 1.41	96	36.76 $\pm$ 1.40	0.16
≥ 40 and <43	155	40.97 $\pm$ 0.79	71	41.00 $\pm$ 0.85	0.82
≥ 43	152	44.77 $\pm$ 1.72	61	44.97 $\pm$ 1.82	0.16
AMH (ng/ml) (median (Q25, Q75))	712	1.60(0.68, 2.92)	302	1.26(0.50, 2.89)	0.11
≤ 1.1	278	0.47(0.20, 0.80)	136	0.41(0.12, 0.78)	0.2
>1.1	434	2.56(1.74, 3.90)	166	2.64(1.70, 4.42)	0.58
Age of spouse (years) (mean $\pm$ SD)	711	40.97 $\pm$ 6.91	295	40.80 $\pm$ 7.27	0.72
Oocyte retrieval	1523		690		
Conventional stimulation		39.86%		38.99%	0.7
Minimal stimulation		60.14%		61.01%	

Table 1: Comparison of the baseline characteristics in administration group and control group.

METHODOLOGY

Study design and intervention methods

The present study comprised Chinese women patients undergoing IVF procedures at Hanabusa Women's Clinic in Japan. The administration of Shawkea DE-T1 by all patients included in our study was considered to be an exposure factor and was voluntarily chosen according to the patients' wishes. Patients who chose to take Shawkea DE-T1 were included in the administration group and daily consumed 300 ml (3 packages) in the 30-34 years-old group, 400 ml (4 packages) in the 35-40 years old group, and 500 ml (5 packages) in women >40 years old, according to the recommendations of the Nutritional Supplement Support Center of Hanabusa Women's Clinic. Patients who chose not to take Shawkea DE-T1 were included in the control group. All patients were treated according to the Japanese Institution for Standardizing Assisted Reproductive Technology (JISART) intervention, with appropriate ovulation-induction programs selected by physicians, and all patients were treated by the same group of embryologists and physicians.

Observation indices and methods

The blastocyst obtained rate of the two groups with different administration durations of Shawkea DE-T1: Successful generation of blastocysts *in vitro* after IVF is directly related to the success of overall IVF treatment in patients. Hence, in this study we observed the blastocyst obtained rates for the patients in the Shawkea DE-T1 administration group and the patients in the control group with different treatment durations, and because the cycle of oocyte/embryo development and maturation was three months, we herein divide the patients to three subgroups of Shawkea DE-T1 administration duration: 1-3 months, 4-6 months, and >6 months.

The blastocyst rate comprised the percentage of patients who obtained at least one blastocyst after IVF treatment as a proportion of the total number of patients in the same group (blastocyst rate = the number of patients who obtained at least one blastocyst during the treatment period / the total number of patients in the same group $\times 100\%$).

Analysis of treatment duration of Shawkea DE-T1 for women at different age levels whose embryos successfully developed to blastocyst stage: A study has shown that female reproductive function declines with age, most notably at the ages of 35, 40 and 43 years [8]. Therefore, for this study we divided patients according to their age: (1) ≥ 30 and <35 years; (2) ≥ 35 and <40 years; (3) ≥ 40 and <43 years; and (4) ≥ 43 years. The actual status of women at different age levels who successfully obtained blastocysts in the administration group was analyzed to provide a reference for the Shawkea DE-T1 administration duration.

Statistical analysis

We analyzed all data using the SPSS 23.0 software package (IBM, Armonk, NY). Measurement data conforming to a normal distribution and showing homogeneous variance are presented as means $\pm$ standard deviation and were compared between two groups using an independent-sample t test. Measurement data did not follow a normal distribution or

Treatment duration	Group	Cases	Blastocyst formation	χ^2	P	OR
1-3 months	Administration group	269	224(83.27)	41.78	1.02×10^{-10}	4.02
	Control group	179	99(55.31)			
4-6 months	Administration group	252	175(69.44)	12.222	4.70×10^{-4}	2.84
	Control group	54	24(44.44)			
>6 months	Administration group	191	142(74.35)	0.093	0.76	1.1
	Control group	69	50(72.46)			

Table 2: Comparison of blastocyst obtained rates in the two groups of IVF-treated women undergoing different treatment durations.

showing heteroscedasticity are presented as medians (Q25, Q75) and were compared between two groups using Mann-Whitney U test. Counting data are presented as numbers of cases and percentages, and were compared using a Chi-squared test. A difference at $P < 0.05$ was considered significant.

RESULTS

Comparison of blastocyst rates in the two groups of patients undergoing different treatment durations

As shown in Figure 1 and Table 2, when we compared their blastocyst production rates, we observed that those patients with 1-3 vs. 4-6 months of treatment durations in the administration groups were significantly higher than in the controls ($P < 0.05$), while we uncovered no difference when the treatment duration was >6 months ($P > 0.05$).

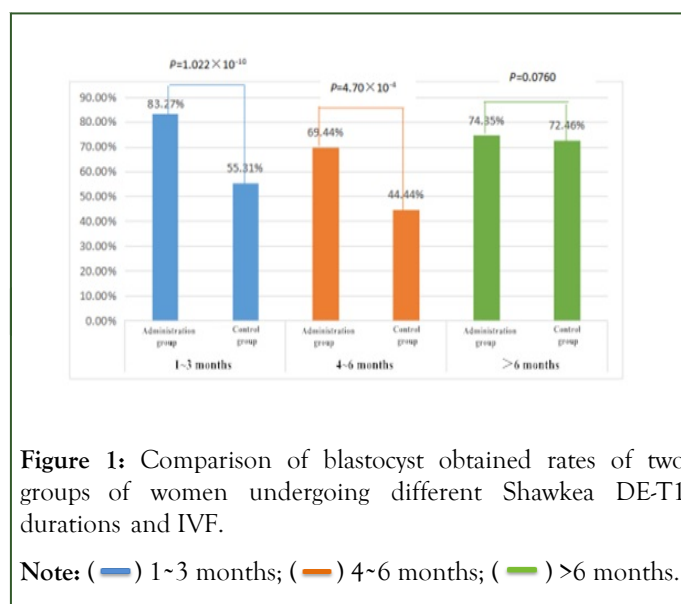


Figure 1: Comparison of blastocyst obtained rates of two groups of women undergoing different Shawkea DE-T1 durations and IVF.

Note: (—) 1~3 months; (—) 4~6 months; (—) >6 months.

Analysis of Shawkea DE-T1 administration duration for women of different ages who generated blastocysts

We also determined in women who produced blastocysts that Shawkea DE-T1 administration duration was increased commensurately with increasing patient age observed in Figure 2 and Table 3.

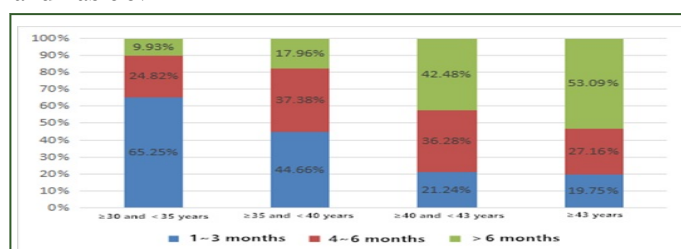


Figure 2: Schematic diagrams showing Shawkea DE-T1 administration durations for women who obtained blastocysts at different age levels.

Note: (—) 1~3 months; (—) 4~6 months; (—) >6 months.

Age	Cases	1-3 months	4-6 months	>6 months
		(n=224)	(n=175)	(n=142)
≥ 30 and <35 years	141	92(65.25)	35(24.82)	14(9.93)
≥ 35 and <40 years	206	92(44.66)	77(37.38)	37(17.96)
≥ 40 and <43 years	113	24(21.24)	41(36.28)	48(42.48)
≥ 43 years	81	16(19.75)	22(27.16)	43(53.09)

Table 3: Shawkea DE-T1 administration durations for IVF-treated women in different age groups who achieved blastocyst formation.

DISCUSSION

Mechanism of Shawkea DE-T1 on reproduction health

From years of clinical observation we discerned that Shawkea DE-T1 administration improved the success rate of IVF-ET. The Follicle-Stimulating Hormone (FSH) receptor is the only gonadotropin receptor present on the ovarian granulosa cell surface during early folliculogenesis, and previous studies have confirmed that Shawkea DE-T1 increased the activities of FSH, luteinizing hormone, and other related reproductive hormones and their receptors on the surfaces of ovarian granulosa cells [5,9]. A previous group showed that Growth Hormone (GH) was also involved in many processes underlying human reproduction. During Oocyte development process, GH could promote cell maturation and increase receptor activity in granulosa cells, and additional research indicates that the effect of GH on reproduction is principally its promotion of Insulin-Like Growth Factor (IGF) secretion [10,11]. IGF is a molecule that effectively promotes granulosa cell activities, and extant studies show that dandelion polysaccharides increase the activity of the IGF-related receptor and also exert blood-glucose lowering, hepatoprotective, and antioxidative effects [1-4]. Our previous investigation revealed that the embryonic implantation rate and live-birth rate after IVF treatment significantly declined with age, and that the decline was more obvious after the age of 40 [8]. Diminished oocyte quality due to aging in women is usually due to meiotic nondisjunction of oocyte chromosomes, resulting in an elevation in the rate of aneuploidy in early embryos and an increase in the rate of spontaneous abortion. The most common autosomal trisomy's, for example, are caused by abnormal chromosomal spindle arrangement and filament-matrix composition during meiosis of oocytes in women of advanced reproductive age [11,12].

The patients who experienced embryo transfer in previous study were 30-46 years old, with a mean age of 36.10 ± 3.97 years [7].

We noted no significant difference in the age of the two groups of patients, and all patients were at a relatively advanced reproductive age. In our previous study we compared the live-birth rate for women with and without Shawkea DE-T1 administration, and our results revealed that the rate for treated women was 57.53%; this was significantly higher than that of the control group at 40.00% ($\chi^2=4.48$, $P=0.045$), and suggested that Shawkea DE-T1 significantly improved the live-birth rate of women treated with IVF [7].

Mechanistically, Shawkea DE-T1 may significantly improve blastocyst obtained rate in patients received IVF treatment, so they have more blastocysts available for embryo transfer. In addition, a study showed that multiple receptor types exist in the ovaries and uterus, and Shawkea DE-T1 can up-regulate the activity of these receptors and thereby balance the secretion of female reproductive hormones [6]. Dandelion polysaccharide also promotes the secretion of GH and affects related receptors [1-4], and play roles in oocyte growth, embryonic implantation, and fetal development. Thus, Shawkea DE-T1 may improve the quality of oocytes during IVF and promote the production of high-quality, fertilized oocytes.

Combining the above functions with the results of our previous and current findings, we hypothesize that Shawkea DE-T1 significantly increases blastocyst obtained rate in women undergoing IVF treatment, and its underlying mechanism may be related to promoting the secretion of GH, thereby increasing the numbers of granulosa cells, inhibiting the decline in granulosa cells, and promoting ovulation. GH actions may involve multiple organs, pathways, and targets including hypoglycemia, antioxidation, and hepatoprotection.

Therefore, the improvement in live-birth rate with Shawkea DE-T1 administration observed in our previous trail may be achieved *via* its two major effects: increasing the overall number of blastocysts and improving blastocyst quality. Given the complex physiologic processes involved in reproduction, further research is necessary to delineate the multi-target synergistic mechanisms of Shawkea DE-T1 on different organs of the hypothalamic-pituitary-ovarian-uterine axis.

Effects of different Shawkea DE-T1 administration durations on the blastocyst obtained rate in women

With the continuous development of ART, blastocyst culture has become more widely employed. Compared with primary embryos, the transfer of embryos grown to the blastocyst stage improves the synchronization of embryos with endometrial receptivity, increases *in vitro* screening of embryos, and achieves favorable pregnancy and implantation rates [13,14]. Blastocyst culture effectively eliminates embryos with poor potential or that show chromosomal abnormalities during their development from cleavage to blastocyst stage, thereby improving the pregnancy outcome with blastocyst transfer [15-17]. Blastocyst transfers have increased globally over recent years and currently account for over 50% of all embryo transfers [18,19]. Hence, in this study we focused on the effect of different administration durations of Shawkea DE-T1 on *in vitro* blastocyst obtained rate in women undergoing IVF treatment. The blastocyst obtained rate reflected the percentage of patients who ultimately obtained at least one blastocyst after treatment relative to the total number of patients in the same group, suggesting that these patients achieved the opportunity to transfer blastocysts and that they exhibited a higher pregnancy rate than patients undergoing transfer of early embryos. Blastocyst obtained rate was thus an important predictor of the treatment outcomes with IVF.

Due to the ethics involved and a respect for honoring patient wishes, we did not artificially create an observation period but only conducted dosage guidance and follow-ups on the Shawkea DE-T1 administration conditions. A majority of the patients in the administration group started Shawkea DE-T1 administration 1-3 months before oocyte retrieval, and the administration duration of Shawkea DE-T1 was determined by the patients according to the results of oocyte retrieval and embryo culture. Under normal circumstances, patients with difficulties in oocyte retrieval and blastocyst culture underwent multiple oocyte retrievals and experienced relatively long treatment duration. Thus, we compared the blastocyst rates of two groups of patients undergoing different treatment durations (1-3 months, 4-6 months, and >6 months), and our results showed that the rates of the patients undergoing 1-3 months and 4-6 months of treatment durations in the administration group were significantly higher than those in the control group ($P < 0.05$), suggesting that patients administered Shawkea DE-T1 achieved a higher blastocyst rate during the same treatment duration.

In another perspective, the blastocyst rate of the patients who underwent a 1-3 months Shawkea DE-T1 administration was 83.27% higher than that for patients who received 4-6 months of treatment (69.44%). The blastocyst rates declined with the extension of Shawkea DE-T1 administration durations, and we speculate that this result may be determined by the basic physiologic conditions of the patients in the corresponding treatment durations. The patients showing healthy basic conditions achieved blastocyst formation after 1-3 months of Shawkea DE-T1 administration, while the patients who did not generate a blastocyst required a longer Shawkea DE-T1 administration

period (4-6 months). Given the differences in the basic physiologic conditions of the patients undergoing 1-3 months and 4-6 months of Shawkea DE-T1 administrations, the blastocyst rates between the two groups could not be compared directly.

We posit that the analysis of blastocyst rate should be performed taking three months as treatment duration; however the patients in the administration group of this study experienced various treatment durations (varying from 1-40 months). With the extension in treatment duration the number of patients in each treatment-duration group was relatively small. In addition, the overall data distribution was quite different, and the heterogeneity of the corresponding data was high. For the reasons above, the data over six months was not suitable for statistical analysis. Therefore, the comparison of the blastocyst rates between the two groups of patients undergoing more than six months of treatment showed no significant differences between groups ($P > 0.05$). A study of larger sample size is warranted in the future to provide additional observations.

Optimization of Shawkea DE-T1 administration duration for women at different ages in order to acquire satisfactory therapeutic efficacy

The results of this study revealed that for women who successfully produced a blastocyst, there were differences in Shawkea DE-T1 administration durations for the different age groups. For women ≥ 30 and < 35 years of age, 65.25% patients successfully achieved blastocyst within one to three months of Shawkea DE-T1 administration, and only 9.93% underwent treatment > 6 months to produce a blastocyst. For women ≥ 43 years of age, only 19.75% successfully achieved blastocyst within one to three months of Shawkea DE-T1 administration, while 53.09% required > 6 months. We therefore recommend that Shawkea DE-T1 administration duration be adjusted according to the baseline conditions of the women (including age), and not be generalized. For women who attempt to conceive at an advanced reproductive age, a sufficient Shawkea DE-T1 administration duration may be more conducive to producing a high quality blastocyst upon embryo culture after IVF.

CONCLUSION

In conclusion, in this study we analyzed the influences of different Shawkea DE-T1 administration durations on blastocyst obtained rate, and further assessed the rates with actual Shawkea DE-T1 administration durations in women in different age levels who obtained blastocyst successfully in IVF treatment. Our result indicated that Shawkea DE-T1 administration increased the blastocyst obtained rate for 1-3 months and 4-6 months of treatment ($P < 0.05$). Due to insufficient sampling of the patients who underwent > 6 months of treatment, we did not note any significant differences in blastocyst obtained rate compared with the patients in the control group ($P > 0.05$). Analysis of the treatment durations with Shawkea DE-T1 in women who achieved blastocyst also suggested that women of advanced

reproductive age should extend Shawkea DE-T1 administration duration to achieve better therapeutic efficacy.

Our relatively high mortality rate could be explained by the fact that the paediatric department where the study was carried out does not have an adequate technical platform for the efficient management of these cases of respiratory distress.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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